

STANDARD OPERATING PROCEDURES
DIVISION OF COMPARATIVE MEDICINE
UNIVERSITY OF SOUTH FLORIDA

SOP#: 422

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TITLE: Tumor Resection for Passage from Mice Contaminated with *Corynebacterium bovis*
SCOPE: All Research and Animal Care Staff
RESPONSIBILITY: Veterinary Staff, Facility Manager, Research and Animal Care Staff
PURPOSE: To Outline the Procedures of Proper Collection for Re-implantation of Tumors Derived from Mice Contaminated with *Corynebacterium bovis*

I. PURPOSE

1. To prevent the introduction of an opportunistic bacterial disease that can invalidate research involving mice.

II. RESPONSIBILITY

1. All research and animal care staff are responsible for reading, understanding, and following the procedures described below.
2. Facility Managers ensure implementation of all procedures.
3. The Veterinarians oversee all aspects of animal health and are assisted by all program staff.

III. PROCEDURES

Equipment

1. Sterile nitrile gloves
2. Oxivir in a spray bottle
3. 15-50 mL conical centrifuge tube with sterile tissue culture media plus Penicillin and Streptomycin
4. Oxivir solution sufficient for mouse immersion
5. Small container with lid (e.g.: small yellow top formalin container) for Oxivir submersion of mouse
6. Sterile pipette tip box for sterile saline
7. Several sterile gauze 4"x4" per mouse
8. Tegaderm (or Bio-Occlusive drape of any brand) cut ~3"x5"; 2x per mouse
9. Sterile surgical drape
10. Sterile instruments if harvesting one tumor from one mouse:
 - a. 2x Scissors (4-5 inches in length, sharp-sharp tips)
 - b. 2x Forceps
 - c. 1x small hemostats
 - d. sterile saline (~100ml)
11. Sterile instruments if harvesting more than one tumor from multiple mice:
 - a. Germinator Glass Bead Sterilizer (requires 20 minutes to warm up to sterilization temperature)
 - b. 2-4x scissors (4-5 inches in length, sharp-sharp tips)
 - c. 2-4x forceps

- d. 1-2x small hemostats
- e. Sterile saline (~100ml)

Tumor Harvest

1. The **day prior to** tumor harvest, prep (i.e., clip) the skin over the intended incision. Taking care to not injure the skin.
2. **While double gloved**, transfer the mouse to induction chamber and euthanize the mouse by isoflurane overdose and cervical dislocation.
3. On a benchtop, pour Oxivir solution into the container (i.e., typically 100-150 mL will achieve submersion.)
4. Within the container, **submerge the euthanized mouse in Oxivir, place lid on container.**
5. Allow the euthanized mouse to soak **submerged in the Oxivir for a minimum of 5 minutes.**
6. **Don clean gloves and Tyvek sleeves** and saturate spray them with Oxivir.
7. Remove everything from the inside of the biosafety cabinet (BSC) with the exception of the Germinator Glass Bead Sterilizer.
 - a. **Saturate spray with Oxivir** the inside of the BSC.
 - b. Allow the Oxivir to sit for 5 minutes, then wipe down the inside of the BSC with Oxivir wipes, cleaning **all vertical surfaces first**, including the front transparent sash, followed by the horizontal work surface and the external sash
8. Remove everything from the inside of the biosafety cabinet (BSC) with the exception of the Germinator Glass Bead Sterilizer.
 - a. **Saturate spray with Oxivir** the inside of the BSC.
 - b. Allow the Oxivir to sit for 5 minutes, then wipe down the inside of the BSC with Oxivir wipes, cleaning **all vertical surfaces first**, including the front transparent sash, followed by the horizontal work surface and the external sash.
9. Spray the horizontal work surface of the BSC with Oxivir **again** and allow it to stay moist.
10. Saturate spray with Oxivir and transfer into the BSC the sterile instrument pack, sterile supplies, and conical tubes containing sterile media and wait 5 minutes.
11. Change gloves and sleeves, and spray **new gloves and Tyvek sleeves** with Oxivir.
12. Open the outer wrap of the sterile instrument and supply packs ensuring instruments and supplies are maintained in a sterile manner. Pour sterile saline into the pipette tip box.
13. Don a pair of sterile surgical gloves (i.e., double glove) and spray gloves with Oxivir. Arrange instruments, supplies, bowls, etc. within the BSC.
14. Remove the mouse from the Oxivir. Use sterile gauze to dry the mouse's skin..

15. Lay dried mouse down on side of the Tegaderm/Bio-Occlusive drape, then press second piece down to seal entire mouse between the two films. Press gently along the edges to ensure good contact with skin and press out air.
16. **Away from the tumor site**, securely grasp the Tegaderm/Bio-Occlusive drape and the underlying skin, then using the **first pair of sterile scissors** cut the skin axial and or transverse to the spine. Put down the scissors.
17. Use fine tipped hemostats to blunt dissect the tumor away from the skin until the flap can be pulled completely away from the tumor. Using a new set of forceps, grasp the tumor from underneath and pull gently upwards. Normally the tumor will “pop” out, if not, use the 2nd pair of clean scissors to complete the dissection from the surrounding tissues.
18. Using **aseptic technique**, use this **second pair of sterile scissors and remaining clean forceps** (i.e., no previous contact with skin) to trim any remaining connective tissue away from the tumor.
19. Aseptically, remove the tumor and transfer the tumor to the 15-50 mL conical tube with sterile tissue culture media + Penicillin-Streptomycin.
20. If serially performing this procedure, forceps and scissors should be placed in the glass bead sterilizer for 15 seconds prior to re-contact with tissue. To cool instruments prior to their contact with tumor tissue, dip them in the sterile saline bowl.
21. Re-implantation of tumors derived from mice contaminated with *Corynebacterium bovis* should be conducted in accordance with SOP 412 Rodent Surgery in a separate BSC remote from the area of derivation.

Approved:

Date: